Prostaglandins, Leukotrienes and Essential Fatty Acids 89 (2013) 139–143

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Review

Review of *sn*-2 palmitate oil implications for infant health

Fabiana Bar-Yoseph*, Yael Lifshitz, Tzafra Cohen

Enzymotec Ltd., Sagi 2000 Industrial Park, Kfar Baruch, Israel

ARTICLE INFO

Keywords:
Sn-2 palmitate
Human milk
Structured triglyceride

ABSTRACT

Human milk provides the optimal balanced nutrition for the growing infant in the first months after birth. The human mammary gland has evolved with unusual pathways, resulting in a specific positioning of fatty acids at the outer *sn*-1 and *sn*-3, and center *sn*-2 of the triacylglyceride, which is different from the triglycerides in other human tissues and plasma. The development of structured triglycerides enables mimicking the composition as well as structure of human milk fat in infant formulas. Studies conducted two decades ago, together with very recent studies, have provided increasing evidence that this unusual positioning of 16:0 in human milk triglycerides has a significant role for infant health in different directions, such as fat and calcium absorption, bone health, intestinal flora and infant comfort. This review aims to unravel the relevance of human milk triglyceride *sn*-2 16:0 for intestinal health and inflammatory pathways and for other post-absorption effects.

1. Introduction

Human breast milk provides the optimum nutrition for infants, and is being designed to provide perfectly balanced nutrition to meet the needs of the growing infant in the first months after birth. About 50% of energy in human milk is provided by fatty acids in the milk triglycerides, which themselves are molecules comprised of mixtures of three fatty acids esterified to a glycerol backbone [1]. Triglyceride synthesis, rather than involving random esterification of three fatty acids to glycerol, involves specific positioning of fatty acids at the outer *sn*-1 and *sn*-3, and center *sn*-2 positions of the triacylglyceride. The human mammary gland has evolved with unusual pathways for acylation of fatty acids into triglycerides for secretion in milk, with these pathways resulting in a different triglyceride structure (triglyceride fatty acid arrangement) from the triglycerides in other human tissues and plasma [2], or common dietary fats and oils. This stereospecific positioning of fatty acids in human milk triglycerides involves preferential positioning of the saturated fatty acid palmitic acid (16:0) at the *sn*-2 position, rather than at the *sn*-1,3 positions, as is typical of human tissue and plasma lipids, and vegetable oils common in human diets, and in the fat blends used in the manufacture of infant formula [3]. Studies over the last two to three decades have provided increasing evidence that this usual positioning of 16:0 in human milk triglycerides promotes the absorption of both 16:0 and calcium in term and preterm infants [4–8]. However, the importance of the *sn*-2 positioning of 16:0 in human milk triglycerides is expected to extend beyond saturated fatty acids and calcium absorption, since it also impacts the composition of unesterified fatty acids in the intestinal lumen, and the composition of unesterified fatty acids and *sn*-2 monoglycerides that enter the intestinal enterocyte. Recently, there has been a growing understanding that dietary fatty acids may influence the intestinal microbiome and contribute in complex ways to neurological and immune system development through roles in cell signaling and regulation of gene expression. This paper provides a review of new studies with triglycerides synthesized to contain 16:0 on the *sn*-2 (also termed β-16:0) and 18:1n-9 on the *sn*-1, 3 position to resemble the major 16:0 containing triglyceride species in human milk. Our purpose is to begin to unravel the relevance of human milk triglyceride *sn*-2 16:0 for intestinal health and inflammatory pathways and for other post-absorption effects.

2. Structured triglycerides

Palmitic acid (C16:0) is the major saturated fatty acid in human milk, accounting for 17–25% of the total fatty acids [2]. Equally important, over 70% of 16:0 is esterified at the milk triglyceride *sn*-2 position [2,9]. The major unsaturated fatty acid in human milk is oleic acid (18:1n-9) and this is mostly esterified at the triglyceride *sn*-1,3 (outer) positions, with the result that triglycerides with the structure 18:1n-9—16:0—18:1n-9 are a major triglyceride species in human milk and represent an estimated 11.8% of the total triglyceride species [2]. Early studies addressing the importance of the human milk triglyceride structure compared fat absorption in infants fed human milk with

* Corresponding author.
E-mail address: fabiana@ENZYMOTEC.COM (F. Bar-Yoseph).

0952-3278
http://dx.doi.org/10.1016/j.plefa.2013.03.002

infants fed formulas containing lard (an unusual animal fat in which 16:0 is also high and esterified in the triglyceride *sn*-2 position), and infants fed lard that had been randomized to redistribute 16:0 equally across all three carbons on the triacylglyceride [10]. The latter studies showed that redistributing 16:0 from the *sn*-2 position of the formula triglyceride led to decreased fat absorption. Although two or more vegetable oils can be blended to give the same average amounts of 16:0, 18:1n-9 and 18:2n-6 in an infant formula as in human milk, the stereo-specific arrangement of vegetables oil triglycerides means that the 16:0 will be present almost entirely on the triglyceride *sn*-1,3 positions [3]. The development of structured triglycerides enables mimicking both the composition as well as the structure of human milk fat for infant formulas. Structured TG are achieved through an enzymatic process by which the 16:0—18:1n-9—16:0 is transformed to 18:1n-9—16:0—18:1n-9. The resulting product contains 17–25% palmitic acid with above 40% located at the center *sn*-2 position.

3. Importance of dietary 16:0 positioning in triglycerides for fatty acid and calcium absorption.

Studies done several decades ago have demonstrated the greater efficiency of fat absorption and softer stools in breast-fed infants compared to that of infants fed with formulas containing 16:0 from saturated vegetables; effect that was linked to the large amounts of 16:0 in human milk at the *sn*-2 position of the milk triglycerides [4–8]. Triglyceride digestion by endogenous lipases leads to hydrolysis of fatty acids from the triacylglyceride *sn*-1,3 linkages, to release two unesterified fatty acids and one *sn*-2 monoglyceride from each triglyceride into the intestinal lumen [11]. A role for the milk bile salt-stimulated lipase in completing hydrolysis of *sn*-2 monoglycerides with 16:0 released during triglyceride digestion is unlikely, since unesterified 16:0 is poorly absorbed [3]. Structuring 16:0 on the triglyceride *sn*-2 position of milk or formula fats improves 16:0 absorption [12,13], and plasma chylomicron triglycerides of breast fed infants are high in *sn*-2 16:0 [14,15]. In addition to low intraluminal solubility, unesterified 16:0 has an increased tendency to combine with divalent cations, such as calcium, to form insoluble soaps, which are malabsorbed [16]. Clinical evidence for this has been provided by studies to show increased fecal excretion of fatty acid soaps of 16:0 and calcium, accompanied by harder stools, in infants fed formula containing 16:0 from saturated vegetable oils rather than structured triglycerides containing β-16:0 [4–8]. Fig. 1 shows the correlation between the level of 16:0 in the milk or formula triglyceride sn-2 position and infant fatty acid and calcium absorption calculated as a modified Cohen's effect size (f2) [17] using data from published studies with term [5–7] and preterm infants [4,8]. Since an effect size of over 0.8 is recognized as a large effect [17], β-16:0 with over 40% 16:0 at the *sn*-2 position would have a large beneficial effect on fatty acid and calcium. The results show that progressively increasing 16:0 at the *sn*-2 (and decreasing 16:0 at the *sn*-1,3 positions) of the formula triglyceride leads to a dose-dependent increase in 16:0 and calcium absorption ($r=0.95$ and $r=0.78$ for 16:0 and calcium, respectively). The reduction in fecal calcium and 16:0 as calcium soaps is accompanied by a decrease in the incidence of harder stools [6,18,19].

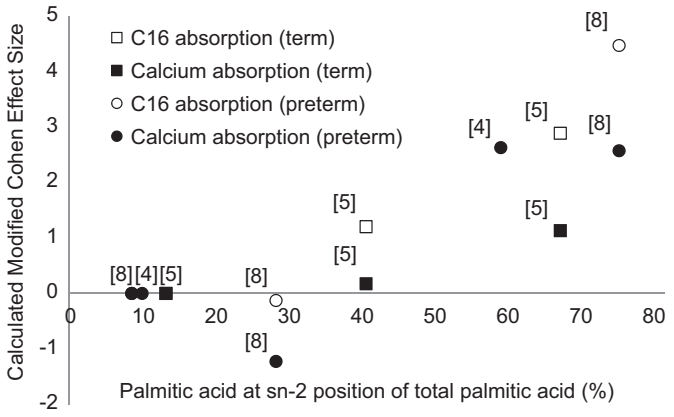

Fig. 1. Modified Cohen's effect size of the effect of the 16:0 position in formula triglycerides on the absorption of 16:0 and calcium in term and preterm infants. The percentage of 16:0 in formula triglycerides (*x*-axis) was plotted against the mean percentage of 16:0 absorption (open circles or squares) or calcium (solid circles or squares) reported in clinical studies with preterm (circles) and term (squares) infants to derive the Cohen effect (f2), as described (Cohen 1988) [17]. The figure shows that progressive enrichment of 16:0 at the *sn*-2 rather than *sn*-1,3 positions of formula triglycerides leads to a dose response increase in calcium and 16:0 absorption. The numbers on the data points on the graph refer to the published studies, as cited in the references.

4. Bone health

Malabsorption of calcium in fatty acid soaps in infants fed formulas containing 16:0 rich vegetable oils has led to interest in the possible effects of milk and formula 16:0 on bone mineralization in young infants [6]. Recent advances in methods for assessment of bone strength parameters with the development of quantitative measures of bone using supersonic speed of sound (SOS) have recently been applied to this direction of research. Ultrasound bone sonometry is a non-invasive technique that enables quantitative longitudinal assessment of changes in bone parameters in the tibia or other bones in term and preterm infants from birth [20–22]. Litmanovitz et al. recently applied the bone SOS technology in a randomized, controlled, double-blind clinical study of bone parameters in term infants fed formula containing triglycerides with *sn*-2 16:0 from InFat® or standard vegetable oil blends with comparison to a non-randomized group of breast-fed infants [23]. Tibia bone SOS decreased during the first 3 months

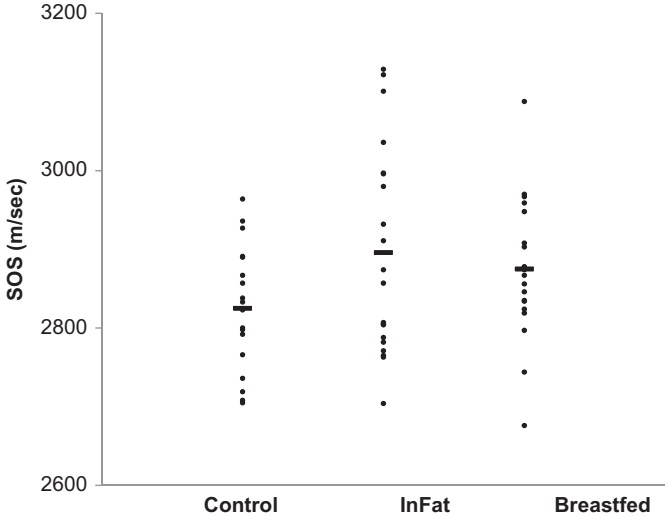

Fig. 2. Ultrasound Speed of Sound (SOS) of the tibia of term infants 3 fed with formula containing 16:0 in structured triglycerides (β-16:0) or unmodified vegetable oil, or who were breast-fed. from birth to 12 weeks of age. Data for each infant within each group is shown by the individual points. The group mean is indicated by the solid line, with the mean for the group fed β-16:0 ($n=20$) significantly higher than for the group fed the standard formula ($n=18$) ($p<0.05$), and not different from the group of the breast-fed infants ($n=22$). The formulas contained about 20% 16:0, with 43% or 14% 16:0 in the *sn*-2 position of the β-16:0 and standard formulas, respectively.

after birth in all infants, consistent with the findings of studies using SOS to assess bone in term and preterm infants [24,25]. However, this recent study showed that infants fed 16:0 as β-16:0 had significantly higher bone SOS, at about 3 months of age than in infants fed formula with a standard vegetable oil blend (Fig. 2). The bone SOS measures for infants fed the β-16:0 formula were also comparable to those of the group of breast-fed infants [23]. These data confirm and extend studies by Kennedy et al. [6] who over a decade ago used dual-energy X-ray absorptiometry (DEXA) to show higher body bone mass in infants after 12 weeks of feeding with formula containing structured triglycerides enriched in *sn*-2 16:0 rather than a conventional formula. In contrast, Zuccotti et al. [26] recently reported no difference in left tibia bone SOS between exclusively breast-fed ($n=25$) and formula fed ($n=12$) infants at 4 months of age, or when assessed later at 12 months of age; however, the type of formula fed was unspecified. Clearly, more studies are needed to assess both the early and potential longer-term implications of the impact of dietary triglyceride composition and structure on bone mineralization and characteristics in infants.

5. Intestinal health

Knowledge that triglyceride digestion by endogenous lipases leads to release of *sn*-2 monoglycerides and unesterified fatty acids [16], and that the triglyceride structure of human milk and infant formula influences the composition of fatty acids excreted in stools raises the question of whether the composition of *sn*-2 monoglycerides and unesterified fatty acids in the lumen, or absorbed into the intestinal enterocytes, also impacts intestinal development and health. The intraluminal environment is characterized by a complex community of microorganisms, which in number is far in excess of the eukaryotic cells of the human body [27]. The intestinal microflora is an essential "organ" which serves numerous important functions, including protection against pathogens and modulation of inflammatory and immune responses, provision of metabolic intermediates and some vitamins, and regulation of intestinal epithelial proliferation and intestinal maturation [27–29]. Of importance, the fetal intestine is essentially a sterile environment that becomes colonized at birth, with the mode of infant delivery, enteral feeding with human milk or formula, and other environmental factors known to impact early intestinal colonization [30]. Growing understanding that the composition of intestinal microbiotica is influenced by factors in the milk diet of the young infant and that different types of microbiotica are associated with an increased or decreased risk of certain diseases, including allergies, late-onset autism, and inflammatory bowel disease [27] has led to recent interest in the possible role of milk triglyceride structures on the intestinal microflora of young infants. In a recent clinical study, we found that infants fed formula containing 16:0 as β-16:0 from InFat® had higher numbers of *Lactobacilli* and *Bifidobacteria* after 6 weeks of feeding than infants fed a control formula with 16:0 from usual vegetable oils [31]. Notably, the beneficial effects of β-16:0 in increasing *Lactobacilli* and *Bifidobacteria* were also present in the subgroup of infants born by Cesarian section, as well as in the subgroup of infants born vaginally. *Lactobacilli* and *Bifidobacteria* have been associated with promotion of gut maturation and integrity, antagonism against pathogens, and immune modulation [32,33]. Considerable opportunities exist for further research to understand the role of the composition of fatty acids in contributing to the composition of colonizing microflora in the young infant's intestinal lumen.

In this regard, other recent experimental study has used the Muc2 deficient mice to address the possible role of milk 16:0 content and positioning in triglycerides on intestinal inflammation. Muc2 deficient mice ($Muc2^{-/-}$) lack mucin2, which is a major component of the mucus layer that separates and provides a physical barrier for the intestinal epithelial cells from the intraluminal contents [34], protecting the underlying epithelium against luminal substances and microbes [35–37]. The deficiency of mucins in the $Muc2^{-/-}$ mice affects the protective capacities of the mucus layer [38], and as a consequence, bacteria are in direct contact with the intestinal epithelial cells [39]. This in turn leads to the development of spontaneous colitis in $Muc2^{-/-}$ mice, a well-described animal model of enterocolitis [40–42]. In a study with $Muc2^{-/-}$ mice, mice fed 16:0 as InFat® demonstrated a lower extent of intestinal erosions and morphological damage than mice fed the same amount of 16:0 in an unmodified standard vegetable oil blend [43]. Further studies will be needed to address the mechanisms through which β-16:0 (or unesterified 16:0) affects this response in $Muc2^{-/-}$ mice, as well as the possible role of effects mediated by changes in the intestinal microflora, or their metabolism.

6. Infant behavior

Early infant crying is considered to reflect basic, instinctive responses governed by neurochemical mechanisms similar to those that control feeding and drinking (i.e., spontaneous behaviors) (for a review, see Ref. [44]). Early infant crying follows a typical pattern over the day, with about 40% of crying episodes occurring between 16:00 and 22:00 h; it is only after the third month from birth that crying episodes become more distributed throughout the day [45,46]. The developmental regulation of crying coincides with the development of the circadian rhythm and forms in part the neurobiological/neuroendocrine basis for the link between crying behavior and the development of circadian rhythms. Crying behavior in young infants is, therefore, considered as modifiable by endogenous/exogenous stimuli that alter neurochemistry. However, this is complex since early infant crying includes not only spontaneous endogenous crying but also crying due to distress, such as separation from an individual to which the infant has become attached, hunger or other physical distress. Not unexpectedly, the duration of crying in very young infants is inversely correlated with the duration of sleep time [46,47].

In a recent open label study, the percent of daily time spent sleeping was not significantly different between the term gestation infants fed formula with β-16:0 and breast fed infants at 6 weeks (68.4% and 69.5% respectively) or 12 weeks of age (64.3% and 67.1% respectively) (unpublished data). Savino et al. (2006) provided the first evidence that triglycerides enriched in β-16:0 might impact infant crying. In this study, term gestation infants fed a formula containing partially hydrolyzed whey proteins, prebiotic oligosaccharides, and high 16:0 as β-16:0 (41% of the formula 16:0 in the triglyceride *sn*-2 position) showed significantly reduced crying compared to infants fed a control formula without β-16:0 hydrolyzed whey proteins or oligosaccharides [49]. However, the beneficial effects on crying in this study cannot be specifically attributed to the β-16:0. More recently, we found that among term infants fed formula with β-16:0 for the 12 weeks after birth there was a decreased percentage of infants that cried, and lower crying duration during the day and night, and especially in the evening and night hours, when compared to infants fed a standard formula with a similar ~20% 16:0 but from unmodified vegetable oil (Fig. 3). This difference in crying pattern between infants fed β-16:0 and those fed standard formula containing 16:0 from unmodified vegetable oils should probably be attributed to complex of mechanisms.

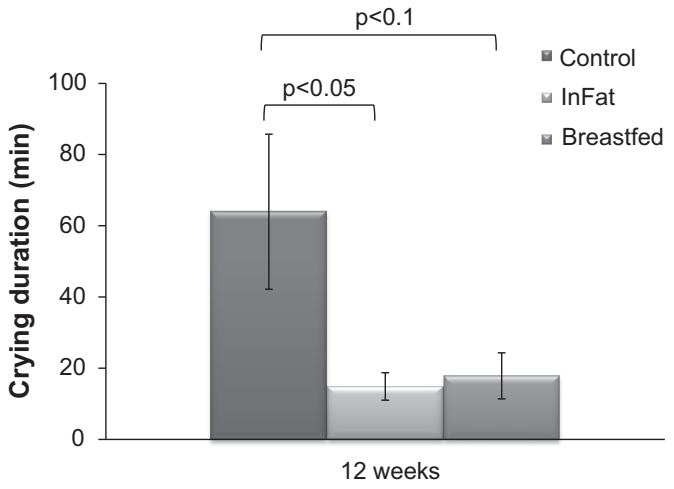

Fig. 3. Total daily crying duration at 12 weeks postnatal. Crying was evaluated by calculating the mean crying duration per day based on parents' reports of crying periods of more than 5 min. At 12 weeks BF infants cried less time during the day compared to control (17.9 ± 6.5 vs. 64.0 ± 21.8 min. p=0.063). InFat formula fed infants also tended to cry less compared to control infants (p=0.047).

Several plausible mechanisms might link milk or structured triglycerides rich in *sn*-1,3 18:1n-9 and *sn*-2 16:0 to altered spontaneous crying in the first few weeks after birth. Structured triglycerides composed of *sn*-1,3 18:1n-9 and *sn*-2 16:0 will lead to uptake of unesterified 18:1n-9 and *sn*-2 16:0 into the intestinal enterocytes [16]. Several acylated molecules, such as the acyl ethanolamines and acylglycines, including palmitoyl and oleoyl ethanolamide are known to be potent signal molecules of the endocannabinoid system that contribute to regulation of relevant physiological processes, such as sleep and pain sensitivity [50], are thought to be involved in the circadian rhythm [51]. Notably, the endogenous opiatergic system is also known to be involved in spontaneous crying (for review see [44]). Interestingly, the developmental regulation of crying coincides with the development of the circadian rhythm. This is perhaps a key, whereby alterations of circadian rhythm development or neuro-endocrine mechanisms therein may relate dietary variables that perturb these systems to crying behavior (see for e.g. the work of [52]. Lower crying behavior in the late afternoon among infants fed formula with β-16:0 is consistent with a neurochemical mechanism, interfacing with the development of circadian rhythm and limbic inhibition of spontaneous crying regulated via brainstem mechanism. Melatonin and fatty acid ethanolamides, including oleoyl ethanolamide, are possible targets for considerations as mediators of the effects of formula fats. Of interest, Banni et al. recently reported that feeding β-16:0 altered the endocannabinoid system and feed efficiency in post-weaning rats [53], suggesting that structured triglycerides may have effects on multiple physiological regulatory processes in young infants.

7. Summary

Research on the physiological importance to young infants of the unusual triglyceride structures in human milk, specifically the preferential acylation of 16:0 at the *sn*-2 position, with large amounts of 18:1n-9 at the *sn*-1,3 positions, is seeing regrowth of interest, made possible in part by technological advances which now make it possible to synthesize dietary triglycerides with the structure 18:1n-9—16:0—18:1n-9. Early work linked the enrichment of 16:0 at the *sn*-2 position of human milk triglycerides to a high efficiency of fatty acid absorption, prevention of calcium malabsorption and softer stools in breast-fed infants. Recent studies are now confirming and extending these observations to show that the *sn*-2 16:0 structure with 18:1n-9 on the triglyceride *sn*-1,3 positions also increases early bone mineralization and development, influences the composition of the intestinal microflora, may lower the extent and severity of intestinal inflammation in response to insult, and may also have neurobiological effects that include modulation of early infant crying. As discussed in this review of recent studies, the effect of triglycerides enriched in *sn*-2 16:0, and of β-16:0 itself is likely to extend well beyond fatty acid and mineral absorption, although much remains to be learnt regarding biological mechanism and potential implication for infant nutrition.

Acknowledgments

We thank Prof. Sheila Innis from the Child and family Research Institute, Vancouver, Canada for the scientific support and critical review of this manuscript.

References

[1] M. Giovannini, E. Riva, C. Agostoni, Fatty acids in pediatric nutrition, Pediatr. Clin. North Am. 42 (4) (1995) 861–877.
[2] W.C. Breckenridge, L. Marai, A. Kuksis, Triglyceride structure of human milk fat, Can. J. Biochem. 47 (8) (1969) 761–769.
[3] R.G. Jensen, Comments on the extraction of fat from human milk for analysis of contaminants, Chemosphere 31 (9) (1995) 4197–4200. (author reply 200-5).
[4] V.P. Carnielli, I.H. Luijendijk, J.B. van Goudoever, et al., Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: effects on fat and mineral balance, Am. J. Clin. Nutr. 61 (5) (1995) 1037–1042.
[5] V.P. Carnielli, I.H. Luijendijk, J.B. Van Goudoever, et al., Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance, J. Pediatr. Gastroenterol. Nutr. 23 (5) (1996) 553–560.
[6] K. Kennedy, M.S. Fewtrell, R. Morley, et al., Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics, and bone mineralization, Am. J. Clin. Nutr. 70 (5) (1999) 920–927.
[7] A. Lopez-Lopez, A.I. Castellote-Bargallo, C. Campoy-Folgoso, et al., The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces, Early Hum. Dev. Suppl. S83–94 (2001) 65.
[8] A. Lucas, P. Quinlan, S. Abrams, S. Ryan, S. Meah, P.J. Lucas, Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants, Arch. Dis. Child Fetal Neonatal Ed. 77 (3) (1997) F178–F184.
[9] R.G. Jensen, Lipids in human milk, Lipids 34 (12) (1999) 1243–1271.
[10] L.J. Filer Jr., F.H. Mattson, S.J. Fomon, Triglyceride configuration and fat absorption by the human infant, J. Nutr. 99 (3) (1969) 293–298.
[11] H. Mu, C.E. Hoy, The digestion of dietary triacylglycerols, Prog. Lipid Res. 43 (2) (2004) 105–133.
[12] E.L. Lien, The role of fatty acid composition and positional distribution in fat absorption in infants, J. Pediatr. 125 (5 Pt. 2) (1994) S62–S68.
[13] E.L. Lien, R.J. Yuhas, F.G. Boyle, R.M. Tomarelli, Corandomization of fats improves absorption in rats, J. Nutr. 123 (11) (1993) 1859–1867.
[14] V.P. Carnielli, I.H. Luijendijk, R.H. van Beek, G.J. Boerma, H.J. Degenhart, P.J. Sauer, Effect of dietary triacylglycerol fatty acid positional distribution on plasma lipid classes and their fatty acid composition in preterm infants, Am. J. Clin. Nutr. 62 (4) (1995) 776–781.
[15] S.M. Innis, R. Dyer, Dietary triacylglycerols with palmitic acid (16:0) in the 2-position increase 16:0 in the 2-position of plasma and chylomicron triacylglycerols, but reduce phospholipid arachidonic and docosahexaenoic acids, and alter cholesteryl ester metabolism in formula-fed piglets, J. Nutr. 127 (7) (1997) 1311–1319.
[16] S.M. Innis, Dietary triacylglycerol structure and its role in infant nutrition, Adv. Nutr. 2 (3) (2011) 275–283.
[17] J. Cohen, in: J.E. Chappell, M.T. Clandinin, C. Kearney-Volpe, B. Reichman, P.W. Swyer (Eds.), Statistical power analysis for the behavioral sciences, second ed.,Lawrence Earlbaum Associates, Hillsdale, NJ, 1988.
[18] P.T. Quinlan, S. Lockton, J. Irwin, A.L. Lucas, The relationship between stool hardness and stool composition in breast- and formula-fed infants, J. Pediatr. Gastroenterol. Nutr. 20 (1) (1995) 81–90.
[19] A. Sidnell, E. Greenstreet, Infant nutrition: review of lipid innovation in infant formula, Nutr. Bull. 36 (3) (2011) 373–380.
[20] A.J. Foldes, A. Rimon, D.D. Keinan, M.M. Popovtzer, Quantitative ultrasound of the tibia: a novel approach for assessment of bone status, Bone 17 (4) (1995) 363–367.

[21] X. Liao, W. Zhang, J. He, J. Sun, P. Huang, Bone measurements of infants in the first 3 months of life by quantitative ultrasound: the influence of gestational age, season, and postnatal age, Pediatr. Radiol. 35 (2005) 847–853.
[22] L. Pereda, T. Ashmeade, J. Zaritt, J.D. Carver, The use of quantitative ultrasound in assessing bone status in newborn preterm infants, J. Perinatol. 23 (8) (2003) 655–659.
[23] I. Litmanovitz, K. Davidson, A. Eliakim, et al. High beta-palmitate formula and bone strength in term infants: a randomized, double-blind, controlled trial. Calcif. Tissue Int. 92 (1) (2013) 35–41.
[24] A. Eliakim, D. Nemet, O. Friedland, T. Dolfin, R.H. Regev, Spontaneous activity in premature infants affects bone strength, J. Perinatol. 22 (8) (2002) 650–652.
[25] I. Litmanovitz, T. Dolfin, O. Friedland, et al., Early physical activity intervention prevents decrease of bone strength in very low birth weight infants, Pediatrics 112 (1 Pt. 1) (2003) 15–19.
[26] G. Zuccotti, A. Vigano, L. Cafarelli, et al, Longitudinal changes of bone ultrasound measurements in healthy infants during the first year of life: influence of gender and type of feeding, Calcif. Tissue Int. 89 (4) (2011) 312–317.
[27] L. Dethlefsen, P.B. Eckburg, E.M. Bik, D.A. Relman, Assembly of the human intestinal microbiota, Trends Ecol. Evol. 21 (9) (2006) 517–523.
[28] A.L. Kau, P.P. Ahern, N.W. Griffin, A.L. Goodman, J.I. Gordon, Human nutrition, the gut microbiome and the immune system, Nature 474(7351) (2011) 327–336.
[29] R. Lodinova, V. Jouja, A. Lanc, Influence of the intestinal flora on the development of immune reactions in infants, J. Bacteriol. (1967) 797–800.
[30] L. Niers, M. Stasse-Wolthuis, F.M. Rombouts, G.T. Rijkers, Nutritional support for the infant's immune system, Nutr. Rev. 65 (8 Pt. 1) (2007) 347–360.
[31] S. Yaron, D. Shachar, L. Abramas, et al., Effect of high beta-palmitate content in infant formula on the intestinal microbiota of term infants: a double-blind, randomized pilot study, J. Pediatr. Gastroenterol. Nutr (2012). (in press).
[32] P.C. Calder, S.K. Etschmann, E.C. de Jong, C. Dupont, Early Nutr. Immun.: Progr. Perspect. 96 (2006) 774–790.
[33] R. Pai, G. Kang, Microbes in the gut: a digestable account of host-symbiont interactions, Indian J. Med. Res. 128 (5) (2008) 587–594.
[34] A. Velcich, L. Palumbo, L. Selleri, G. Evans, L. Augenlicht, Organization and regulatory aspects of the human intestinal mucin gene (Muc2) locus, J. Biol. Chem. 272 (12) (1997) 7968–7976.
[35] G. Hecht, Innate mechanisms of epithelial host defense: spotlight on intestine, Am. J. Physiol. 277 (3 Pt. 1) (1999) C351–C358.
[36] P. Dharmani, V. Srivastava, V. Kissoon-Singh, K. Chadee, Role of intestinal mucins in innate host defense mechanisms against pathogens, J. Innate Immun. 1 (2) (2009) 123–135.
[37] M.A. Hollingsworth, B.J. Swanson, Mucins in cancer: protection and control of the cell surface, Nat. Rev. Cancer 4 (1) (2004) 45–60.
[38] M.E. Johansson, D. Ambort, T. Pelaseyed, et al., Composition and functional role of the mucus layers in the intestine, Cell. Mol. Life Sci. 68 (22) (2011) 3635–3641.
[39] M.E. Johansson, M. Phillipson, J. Petersson, A. Velcich, L. Holm, G.C. Hansson, The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria, Proc. Natl. Acad. Sci. U.S.A. 105 (39) (2008) 15064–15069.
[40] A. Velcich, W. Yang, J. Heyer, et al., Colorectal cancer in mice genetically deficient in the mucin Muc2, Science 295 (5560) (2002) 1726–1729.
[41] M. Van der Sluis, B.A. De Koning, A.C. De Bruijn, et al., Muc2-deficient mice spontaneously develop colitis, indicating that Muc2 is critical for colonic protection, Gastroenterology 131 (1) (2006) 117–129.
[42] P. Lu, N. Burger-van Paassen, M. van der Sluis, et al., Colonic gene expression patterns of mucin Muc2 knockout mice reveal various phases in colitis development, Inflamm. Bowel Dis. (2011).
[43] Y. Lifshitz, P. Lu, F. Bar-Yoseph, et al, In: Proceedings of ISSFAL, Vancouver, Canada, 2012.
[44] J.D. Newman, Neural circuits underlying crying and cry responding in mammals, Behav. Brain Res. 182 (2) (2007) 155–165.
[45] I. St. James-Roberts, J. Hurry, J. Bowyer, Objective confirmation of crying durations in infants referred for excessive crying, Arch. Dis. Child. 68 (1) (1993) 82–84.
[46] I. St. James-Roberts, T. Halil, Infant crying patterns in the first year: normal community and clinical findings, J. Child Psychol. Psychiatry 32 (6) (1991) 951–968.
[47] J. Kirjavainen, L. Lehtonen, T. Kirjavainen, P. Kero, Sleep of excessively crying infants: a 24-hour ambulatory sleep polygraphy study, Pediatrics 114 (3) (2004) 592–600.
[49] F. Savino, E. Palumeri, E. Castagno, et al., Reduction of crying episodes owing to infantile colic: a randomized controlled study on the efficacy of a new infant formula, Eur. J. Clin. Nutr. 60 (11) (2006) 1304–1310.
[50] J.M. Walker, J.F. Krey, C.J. Chu, S.M. Huang, Endocannabinoids and related fatty acid derivatives in pain modulation, Chem. Phys. Lipids 121 (1–2) (2002) 159–172.
[51] L.K. Vaughn, G. Denning, K.L. Stuhr, H. de Wit, M.N. Hill, C.J. Hillard, Endocannabinoid signalling: has it got rhythm? Br. J. Pharmacol. 160 (3) (2010) 530–543.
[52] R.G. Barr, M.S. Kramer, I.B. Pless, C. Boisjoly, D. Leduc, Feeding and temperament as determinants of early infant crying/fussing behavior, Pediatrics 84 (3) (1989) 514–521.
[53] S. Banni, G. Carta, E. Murru, et al. In: Proceedings of ISSFAL, Vancouver, Canada, 2012.